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Scope of Research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, and model studies on the action of ion channels. In addition, artificial designed peptides have also been developed as useful tools in molecular biology and potentially in human medicine.

Research Activities (Year 2002)

Presentations

Influence of amino acid numbers between two ligand cysteines of zinc finger proteins on affinity and specificity of DNA binding, Nagaoka M, Kondo Y, Sugiura Y, Annual meeting, Pharm. Soc. Jpn., 27 March.

Design and function of Cys_2His_2-type zinc finger proteins, Sugiura Y, 29th symposium on biological molecular sciences, 12 July.

Design of novel zinc finger peptide recognizing complementary strand, Shiraishi Y, Nagaoka M, Sugiura Y, 6th European conference on bioinorganic chemistry, 1 August.

Engineering of a novel metalloprotein with the conserved residues of Cys_2His_2-type zinc finger, Hori Y, Sugiura Y, 6th European conference on bioinorganic chemistry, 1 August.

Oligoarginine-mediated delivery of bioactive peptides into cells, Futaki S, Nakase I, Niwa M, Suzuki T, Nameki D, Kodama E, Matsuoka M, Sugiura Y, 39th Japanese peptide symposium, 17 October.

Grants

Sugiura Y, Development of order-made type artificial restriction enzymes and repressors, Grant-in-Aid for University and Society Collaboration, 1 April 2000 - 31 March 2003.

Sugiura Y, Regulation of cellular gene function by novel DNA bending finger, Grant-in-Aid for Scientific Research (B) (2), 1 April 2001 - 31 March 2004.

Sugiura Y, Role of multi-zinc fingers in gene expression and creation of their architectures, Grant-in-Aid for Scientific Research (B) (2), 1 April 2002 - 31 March 2005.

Futaki S, Design of membrane-current regulatory systems using assembly modulation of transmembrane peptides by extramembrane signals,Grant-in-Aid for Scientific Research on the Priority Area of Molecular Synchronization for the Design of New Materials, 1 April 2001 - 31 March 2003.

Futaki S, Creation and intracellular delivery of novel peptides for the regulation of transcription, Grant-in-Aid for Scientific Research (B) (2), 1 April 2000 - 31 March 2003.

Novel Strategy for the Design of New Zinc Finger: Creation of Zinc Finger for AT-Rich Sequence by α-helix Substitution

A novel strategy for the design of a zinc finger peptide on the basis of α-helix substitution has been demonstrated (Figure 1). Sp1HM is a helix-substituted mutant for the wild-type Sp1(zf123) and its α-helix of each finger is replaced by that of fingers 4-6 of CF2-II. The circular dichroism spectrum of Sp1HM suggests that Sp1HM has an ordered secondary structure similar to Sp1(zf123). From the analyses of the DNA binding affinity and specificity by gel mobility shift assay, it is clearly indicated that Sp1HM specifically binds to the AT-rich sequence (5'-GTA TAT ATA-3') with 3 nM dissociation constant (Figure 2). Moreover, the zinc finger peptides for the sequence alternating between the AT- and GC-rich subsites can also be created by the α-helix substitution. This strategy is evidently effective and is also more convenient than the phage display method. Consequently, our design method is widely applicable to creating zinc finger peptides with novel binding specificities.

M. Nagaoka, Y. Doi, J. Kuwahara, Y. Sugiura, *J. Am. Chem. Soc.*, **124**, 6526-6527 (2002).

Figure 1

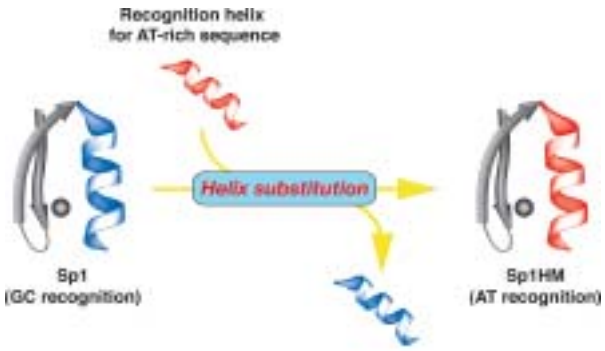
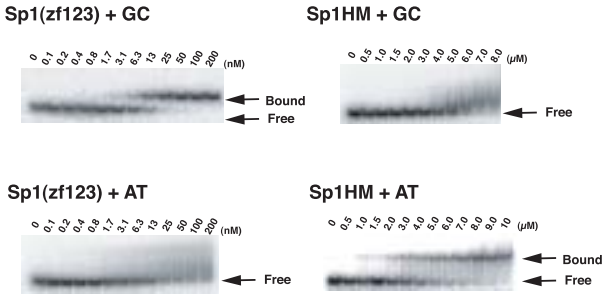
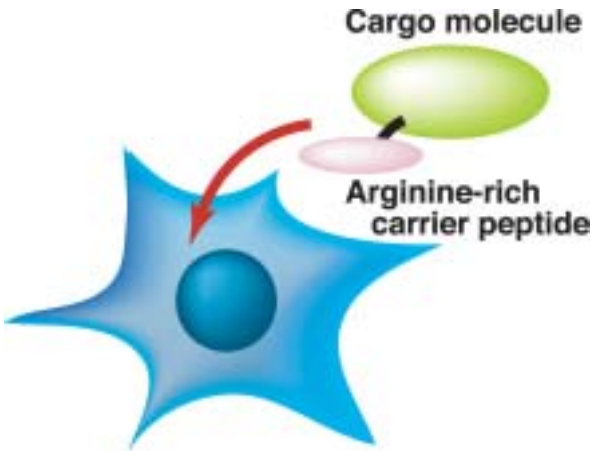


Figure 2



Basic-Peptide Mediated Protein Delivery into Living Cells

A basic peptide derived from HIV-1 Tat has been reported to have the ability to translocate through the cell membranes and to bring exogenous proteins into the cells. We have demonstrated that these features were observable among many arginine-rich peptides including octaarginine $(Arg)_8$, and the presence of a ubiquitous internalization mechanism for arginine-rich oligopeptides has been suggested [1]. Branched-chain arginine peptides were also found to translocate through the cell membranes, which suggested the importance of arginine cluster for the internalization [2]. Among the branched-chain peptides examined, the peptide having eight arginine residues showed the most efficient translocation. The charge-dependent way of internalization was basically similar to that observed for the linear oligoarginine peptides. The above findings would provide new insights on the translocation of arginine-rich peptides and on the design of carrier peptides for intracellular protein delivery.



1. T. Suzuki, S. Futaki, M. Niwa, S. Tanaka, K. Ueda, Y. Sugiura, *J. Biol. Chem.*, **277**, 2437-2443 (2002).
2. S. Futaki, I. Nakase, T. Suzuki, Y. Zhang, Y. Sugiura, *Biochemistry*, **41**, 7925-7930 (2002).